
Bio-Scale™ Mini
Macro-Prep® High Q,
High S and DEAE
Cartridges, 1 and 5 ml

Instruction Manual

Catalog #

732-4120, 732-4122, 732-4124,
732-4130, 732-4132, 732-4134,
732-4140, 732-4142, 732-4144

BIO-RAD

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Section 1

Introduction

Bio-Scale Mini cartridges have a patent-pending double-wall design that provides extra durability and allows easy, reliable runs with aqueous buffers commonly used for protein purification. The polypropylene luer fittings and internal sealing surfaces ensure leak-free operation at pressures up to 45 psi. Bio-Scale Mini cartridges are convenient, disposable, and supplied ready for use. They are easy to use and prepacked for fast, reproducible chromatographic separations. Cartridges are available for a variety of chromatographic techniques, including desalting, ion exchange, affinity, and mixed-mode chromatography. The design of Bio-Scale Mini cartridges offers:

- Ready-to-go convenience; simply equilibrate the cartridge in the buffer of choice
- Luer fittings for convenient connection to any chromatography system or directly to a Luer-Lok syringe

Bio-Scale Mini Macro-Prep High Q and S and Macro-Prep DEAE cartridges are packed with Macro-Prep® ion exchange media. These media are based on hydrophilic spherical polymeric beads designed for the purification of proteins, nucleic acids, viruses, plasmids, and other macromolecules. Macro-Prep beads are designed to provide medium capacity, low backpressure, and high productivity. Detailed product information is given in Tables 1 and 2. See Ordering Information for a listing of the complete Bio-Scale Mini cartridge product line.

Table 1. Bio-Scale Mini Macro-Prep High Q, High S, and DEAE cartridge specifications.

| | |
|----------------------------|---|
| Sizes | 1 ml and 5 ml bed volumes |
| Dimensions | 1 ml: 40 mm length x 5.6 mm inner diameter 5 ml: 40 mm length x 12.6 mm inner diameter |
| Maximum pressure tolerance | 45 psi |
| Operational flow rates | 1 ml: 1–6 ml/min (240–1460 cm/hr) 5 ml: 1–15 ml/min (50–720 cm/hr) |
| Fittings: | Female luer inlet; male luer outlet |
| Column material | Polypropylene |
| Frit material | Polyethylene (HDPE) |
| Shipping conditions | 20% ethanol |
| Storage recommendations | 20% ethanol |
| Autoclavability | Not autoclavable |

Table 2. Macro-Prep High Q & S and Macro-Prep DEAE specifications.

| Properties | High Q | High S | DEAE |
|---|------------------------------|------------------------------|------------------------------|
| Type of ion exchanger | Strong anion | Strong cation | Weak anion |
| Functional group | $-N^+(CH_3)_3$ | $-SO_3^-$ | $-N^+(C_2H_5)_2$ |
| Total ionic capacity | $400 \pm 75 \mu\text{eq/ml}$ | $160 \pm 40 \mu\text{eq/ml}$ | $175 \pm 75 \mu\text{eq/ml}$ |
| Dynamic binding capacity* | $\geq 37 \text{ mg BSA/ml}$ | $\geq 49 \text{ mg IgG/ml}$ | $\geq 30 \text{ mg BSA/ml}$ |
| Shipping counterion | Cl^- | Na^+ | Cl^- |
| Chemical stability | | | |
| 1% SDS, 24 hr | Yes | Yes | Yes |
| 6M guanidine-HCl, 24 hr | Yes | Yes | Yes |
| pH stability | 1–10 | 1–12 | 1–10 |
| Total organic carbon in ppm, after 24 hours at 22°C | | | |
| 0.1 M HCl | 0 | 4 | 0 |
| 0.2 M H_3PO_4 | 0 | 13 | 0 |
| 0.01 M NaOH | 28 | N/A | N/A |
| 0.1 M NaOH | 200 | 31 | 26 |
| 1.0 M NaOH | 337 | 49 | 121 |
| Antimicrobial agent | 20% ethanol | 20% ethanol | 20% ethanol |
| Regeneration | 1–2 M NaCl | 1–2 M NaCl | 1–2 M NaCl |
| Storage conditions | 20% ethanol | 20% ethanol | 20% ethanol |

* 10% breakthrough capacity determined in a 1.1 x 20 cm column.

Section 2

Connecting to Bio-Rad's Low-Pressure Chromatography Instruments

Bio-Scale Mini cartridges are ideal for use with Bio-Rad's BioLogic™ LP chromatography system, Econo™ gradient pump, the patented* Model EP-1 Econo™ pump, and all low-pressure chromatography instruments. Bio-Scale Mini cartridges can be conveniently connected directly to the system using the luer fittings on the cartridge.

1. Install 1.6 mm inner diameter (ID) tubing in the pumphead. Adjust the platen pressure screw (on pumphead) – using a screwdriver or coin, turn the screw counterclockwise as far as it will go, then turn clockwise three full turns.

* US patent 5,135,658

Assemble with fittings and lock rings as shown in Figure 1.

(Use orange lock rings and medium size barb fittings with 1.6 mm tubing.)

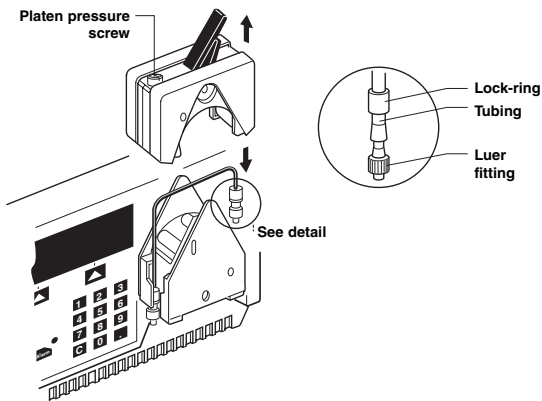


Fig. 1. BioLogic LP system setup.

2. To maximize gradient accuracy and to apply samples efficiently, install 1.6 mm ID tubing from the pump to the MV-6 sample inject valve (if available). If using the MV-6 sample inject valve, turn the knob counterclockwise as far as it will go so it will now correspond to the printed diagram on the valve (see Figure 2).

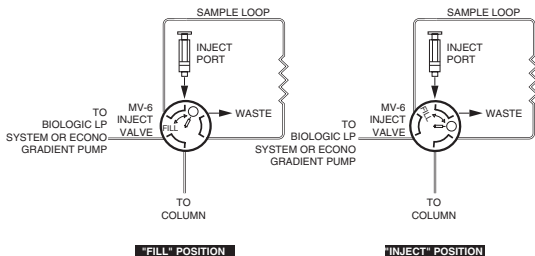


Fig. 2. Connecting to an MV-6 valve.

3. Connect the inlet of the cartridge to the male luer fitting on the MV-6 sample inject valve (see Figure 2). If not using the MV-6 sample inject valve, connect a barb to male luer fitting on the 1.6 mm ID tubing, then connect to the top of the female luer on the Bio-Scale Mini cartridge. For optimum performance, a cartridge should be mounted vertically with the arrow on the cartridge pointing downward (see Figure 3).
4. Connect the cartridge outlet to the 1.6 mm ID tubing leading to the BioLogic LP system optics module or to the Model EM-1 Econo™ UV monitor. It is recommended to use the shortest length (approximately 10 cm) of 1.6 mm ID tubing. Connect a barb to female luer to the 1.6 mm ID tubing, then connect to the bottom of the male luer on the Bio-Scale Mini cartridge.



Fig. 3. Cartridge and fittings.

Section 3

Connecting to Other Liquid Chromatography Systems

Bio-Scale Mini cartridges can be connected to any liquid chromatography system, provided that the maximum pressure limit (3 bar, 45 psi, or 300 kPa) of the cartridges is not exceeded. It is recommended that the system pressure limit be set according to the cartridge pressure limit. Pressures in excess of 3 bar are usually caused by restrictions in tubing or detector cells downstream from the cartridge. Bio-Rad offers two fitting kits for easy connection of a Bio-Scale Mini cartridge to a BioLogic DuoFlow, HPLC, or FPLC-type system.

3.1 BioLogic DuoFlow Systems

The luer to BioLogic system fittings kit (catalog #732-0113) includes 1/4-28 female to male luer and 1/4-28 female to female luer to connect one Bio-Scale Mini cartridge to the BioLogic DuoFlow system, see Figure 4.



Fig. 4. Luer to 1/4-28 adaptor.

3.2 HPLC Systems

The luer to 10-32 adaptor fittings kit (catalog #732-0112) provides fittings necessary to connect the Bio-Scale Mini cartridge to nut and ferrule type fittings found on most HPLC systems. Alternatively, the cartridge can be connected to HPLC systems via a low dead-volume 1/16 inch union with a new piece of stainless-steel tubing attached to the union. Simply slip a short length of the 0.8 mm ID tubing over the 1/16 inch OD stainless-steel tubing to a distance of 1 cm.

3.3 FPLC Systems

The luer to M6 adaptor fittings kit (catalog #732-0111) provides fittings necessary to connect the Bio-Scale Mini cartridge to the M6 fittings found on FPLC or related systems. Alternatively, connection can be made by using one GE Healthcare Union luer lock female to M6 female fitting (GE 18-1027-12) and one female luer lock to M6 male fitting (Upchurch P-686 or GE 18-1027-62). To prevent tubing or cartridge failure, do not exceed the maximum recommended flow rate of the cartridge.

Section 4

Preparing a Cartridge For Use

Bio-Scale Mini Macro-Prep High Q, Macro-Prep High S, and Macro-Prep DEAE cartridges contain 20% ethanol (v/v) as the storage solution. The fully hydrated support is ready to use after equilibrating the cartridge in the buffer of choice. To perform buffer exchange, connect the cartridge to a liquid chromatography system or peristaltic pump and condition it as instructed below:

1. Set pump flow rate to 3.0 ml/min (731 cm/hr) for the 1 ml cartridge or 6.0 ml/min (288 cm/hr) for the 5 ml cartridge.
2. Wash the cartridge with degassed low-salt buffer for 2 min.
3. Wash the cartridge with degassed high-salt buffer for 5 min.

4. Equilibrate the cartridge with low-salt buffer for 5 min.
5. Reduce the flow rate to that which will be used in the purification protocol.

4.1 Sample Preparation

Proper pH and ionic strength are necessary for consistent and reproducible results. Sample can be exchanged into the starting buffer or diluted to the starting buffer's concentration. This can be achieved by diluting the sample to the ionic strength of the starting buffer, dialyzing against the starting buffer, or exchanging it into the starting buffer. Buffer exchange can be accomplished using a Bio-Scale Mini P-6 cartridge, Bio-Spin® 6 or Bio-Spin 30 column, Econo-Pac® 10DG desalting column, or Bio-Gel® P-6DG gel filtration gel (see Table 3). The choice of product will depend on sample volume. All samples should be filtered through a 0.45 µm filter prior to cartridge application.

Table 3. Products for buffer exchange.

| Sample Volume | Recommended Product | Use | Catalog # |
|----------------------|---------------------------------|---------------------------------|------------------|
| 50–100 μ l | Bio-Spin 6 column | Desalting proteins \geq 6 kD | 732-6002 |
| 50–100 μ l | Bio-Spin 30 column | Desalting proteins \geq 30 kD | 732-6006 |
| 100 μ l–3 ml | Bio-Scale Mini P-6 cartridge | Desalting proteins \geq 6 kD | 732-4502 |
| Up to 3 ml | Econo-Pac 10DG desalting column | Desalting proteins \geq 6 kD | 732-2010 |
| Unlimited | Bio-Gel P-6DG gel | Desalting proteins \geq 6 kD | 150-0738 |

4.2 General Purification Protocol

Ion exchange chromatography is usually performed using increasing salt gradients or pH gradients to elute the sample components. For best results and to increase cartridge life, samples and buffers should be degassed and filtered through a 0.45 μ m filter.

Common buffers for cation and anion exchange chromatography are listed in Table 4. An appropriate starting point for purifying samples is a linear

gradient from 0.0 to 0.4 M NaCl spanning 1 to 20 column volumes at 120 cm/hr, or 0.5 ml/min for the 1 ml cartridge and 2.5 ml/min for the 5 ml cartridge. The separation can be optimized by changing the gradient profile. At the end of each run, the cartridge can be regenerated with 1.0 M NaCl followed by starting buffer. Return to the desired flow rate and proceed with the next separation.

4.3 Scaling Up the Separation

For quick scale-up, two or three cartridges of the same type can be connected in series.

Backpressure will increase with cartridges in series, so care should be taken to maintain pressures ≤ 45 psi.

Bio-Scale Mini cartridges are available in 1 ml and 5 ml cartridge formats. The Macro-Prep High Q, High S, and DEAE ion exchange media are also available in larger amounts, from 25 ml bottles to bulk quantities, for scaling up methods developed using the cartridges. Macro-Prep High Q, High S, and DEAE media are fully supported with Regulatory Support Files. In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory scale to process scale.

Table 4. Common buffers for ion exchange chromatography.

| Type of Buffering | Buffer Range |
|--------------------------|---------------------|
| Cation Exchanger | |
| Acetic acid | 4.8–5.2 |
| Citric acid | 4.2–5.2 |
| HEPES | 7.6–8.2 |
| Lactic acid | 3.6–4.3 |
| MES | 5.5–6.7 |
| MOPS | 6.5–7.9 |
| Phosphate | 6.7–7.6 |
| PIPES | 6.1–7.5 |
| Pivalic acid | 4.7–5.4 |
| TES | 7.2–7.8 |
| Tricine | 7.8–8.9 |
| Anion Exchanger | |
| Bicine | 7.6–9.0 |
| Bis-Tris | 5.8–7.2 |
| Diethanolamine | 8.4–8.8 |
| Diethylamine | 9.5–11.5 |
| L-Histidine | 5.5–6.0 |
| Imidazole | 6.6–7.1 |
| Pyridine | 4.9–5.6 |
| Tricine | 7.8–8.9 |
| Triethanolamine | 7.3–8.0 |
| Tris | 7.5–8.0 |

Section 5

Care of the Cartridge

5.1 Regeneration

After each use, the cartridge should be regenerated with the appropriate salt, in most cases 1–2 M NaCl in the presence of buffer. Wash with 2 to 4 column volumes of the buffered high salt solution. This reduces the potential for protein precipitation when selecting acid as a cleaning agent.

5.2 Cleaning

After repeated use, an ion exchange cartridge may require thorough cleaning and regeneration to remove bound contaminants. Acceptable cleaning-in-place (CIP) reagents include 1% acetic acid/1% phosphoric acid with 0.4 M NaCl, up to 30% acetic acid, 1% Triton X-100, up to 70% ethanol or 30% isopropyl alcohol, 8 M urea, and 6 M guanidine-HCl. Any of these agents can be combined in an

appropriate cleaning protocol. As a general guide, we recommend the following:

1. Use high salt buffer for regeneration, as above.
2. For aggregated or precipitated proteins, or when dirty feed stock (e.g., crude lysate) has been used, wash with 3–5 column volumes of 6 M guanidine-HCl or 8 M urea at 100 cm/hr.
3. For lipids or hydrophobically bound contaminants, wash with 0.1% Triton X-100 or 20–70% ethanol or isopropyl alcohol, or 1–30% acetic acid. Use 3–5 column volumes at 100 cm/hr.
4. Remove additional contaminants with 0.4 M NaCl in 1% acetic acid/1% phosphoric acid. Use 3–5 column volumes at 100 cm/hr.
5. If the cartridge is to be used again immediately, wash with 2 column volumes of deionized water and 4–5 column volumes of starting buffer at 100 cm/hr. Check the conductivity and pH of the effluent to verify that the column

is equilibrated in the starting buffer before loading the sample.

5.3 Storage

After washing the cartridges with deionized water, Bio-Scale Mini ion exchange cartridges should be purged and stored with PBS, containing 0.05% NaN_3 , or in 20% (v/v) ethanol solution, and capped for extended storage.

Section 6 Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative as listed on the back cover of our catalog, or in the USA call Technical Support at 1-800-4BIORAD.

Section 7

Ordering Information

Bio-Scale Mini Cartridges*

| Description | 5 x 1 ml | 1 x 5 ml | 5 x 5 ml |
|------------------------------|-----------------|-----------------|-----------------|
| UNOsphere™ Q Support | 732-4100 | 731-4102 | 731-4104 |
| UNOsphere S Support | 732-4110 | 731-4112 | 731-4114 |
| Macro-Prep™ High Q Support | 732-4120 | 732-4122 | 732-4124 |
| Macro-Prep High S Support | 732-4130 | 732-4132 | 732-4134 |
| Macro-Prep DEAE Support | 732-4140 | 732-4142 | 732-4144 |
| Bio-Gel P-6 Support | — | 732-4502 | 732-4504 |
| Affi-PRep® Protein A Support | 732-4600 | 732-4602 | — |
| Profinity™ IMAC Support | 732-4610 | 732-4612 | 732-4614 |
| Affi-Gel® DEAE Blue Support | — | 732-4632 | 732-4634 |
| Affi-Gel Blue Support | — | 742-4642 | 732-4644 |

* Visit www.bio-rad.com/cartridges/ for current information on prepacked cartridges.

- Larger package sizes of media are available for process-scale chromatography. Inquire with your local Bio-Rad representative.

Catalog #
Fittings Kits

Description

| | |
|----------|---|
| 732-0111 | Luer to M6 Adaptor Fittings Kit, includes luer to M6 fitting to connect to an FPLC system |
| 732-0112 | Luer to 10-32 Adaptor Fittings Kit, includes luer to polypropylene/PTFE 10-32 fittings to connect 1 cartridge to an HPLC system |
| 732-0113 | Luer BioLogic System Fittings Kit, includes 1/4-28 female to male luer and 1/4-28 female to female luer to connect 1 cartridge to the BioLogic DuoFlow system |

Section 8

References

1. Gagnon P, Avoiding Instrument-associated Aberrations in Purification Scale-up and Scale-down, BioPharm 10, 42–45 (1997)
2. Harris ELV and Angal S, Protein Purification Methods: A Practical Approach, IRL Press, Oxford (1989)
3. Scopes RK, Protein Purification: Principles and Practice (Second Edition), Springer-Verlag, New York (1987)
4. Snyder LR and Kirkland JJ, Introduction to Modern Liquid Chromatography (Second Edition), Wiley, New York (1979)

FPLC is a trademark of GE Healthcare. Luer-Lok is a trademark of Becton, Dickinson and Co. Triton is a trademark of Union Carbide.

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